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Effects of Fermented Nori (*Pyropia yezoensis*) Liquid Fertilizer on Plant Growth Characteristics and Nutrient Content of Komatsuna (*Brassica rapa* L. var. Wakana Komatsuna) Cultivated in Vermiculite

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Abstract

We conducted plant growth experiments in microbe-free vermiculite to study the effects of four types of fermented seaweed liquid fertilizer (SLF) made from nori (*Pyropia yezoensis*) seaweed on the germination, plant growth characteristics, SPAD value, and nutrient content and uptake of komatsuna (*Brassica rapa* L. var. wakana komatsuna). The four types of fermented nori SLF used in this study were prepared by anaerobic fermentation of unwashed nori (SLF1), aerobic fermentation of unwashed nori (SLF2), anaerobic fermentation of washed nori (SLF3), and aerobic fermentation of washed nori (SLF4). Komatsuna seeds treated with 200-, 300-, and 400-fold dilutions of SLFs exhibited improved relative germination ratios (RGRs) at 3 and 4 days after sowing (DAS). At 4 DAS, the RGRs of seeds treated with 10-, 100-, 200-, 300-, and 400-fold SLF dilutions showed no differential effect. Seeds treated with undiluted SLFs did not germinate by 4 DAS. SLF1 may promote komatsuna seed germination. The nitrogen (N), calcium, magnesium, sodium (Na), and iodine (I) contents of plants treated with SLF1 were significantly increased relative to plants treated with the other SLFs. Moreover, the I and Na contents of plants were significantly increased by foliar spray application of different dilutions of SLF1. However, SLF treatment markedly reduced the shoot dry weight compared with ½-strength modified Hoagland nutrient (MHN) solution, although the same amounts of N and K were applied. SPAD

values of the plants treated with SLFs were significantly higher than those of plants treated with MHN. Foliar treatment with SLFs had no significant effect on plant growth, SPAD value, or uptake of nutrients (except Na) relative to the control, but the I content was increased. Plants treated with SLF1 and SLF2 exhibited the highest Na uptake. Foliar spray treatments with SLF1 resulted in the highest I contents in plants. Based on our results, SLF1 is suitable for use as a liquid fertilizer to promote germination and increase nutrient content in komatsuna. These results need to be followed up in soil experiments in the presence of microbes in the rhizosphere.

Keywords

Nori (*Pyropia yezonensis*), Komatsuna (*Brassica rapa* L), Aerobic and Anaerobic Fermentation, Basal and Foliar Application, Macro-Nutrient Content, I Content

1. Introduction

Fertilizers are the most important input in agricultural production and additional types of fertilizer are needed to achieve higher yields to satisfy the worldwide demand for food. Due to increasing direct and indirect negative effects of chemical fertilizers, especially nitrogen (N), on human health and the environment, farmers are moving to organic production methods and low-input sustainable agriculture as alternatives to chemical-based production methods [1]. Seaweeds are macroscopic marine algae which are found attached to rocky substrates in relatively shallow coastal waters. The use of seaweeds as organic fertilizers in farming is an ancient practice that was common among the Romans and also occurred in Britain, France, Spain, Japan, and China. The use of marine macroalgae as a fertilizer in crop production has a long tradition in coastal areas worldwide. The use of seaweed extracts as organic fertilizers is a viable alternative to the use of chemical fertilizers. Seaweed extracts are valuable organic fertilizer sources because they include growth-promoting hormones, such as indole acetic acid, indole butyric acid, cytokinin, auxin, and abscisic acid, as well as trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins, and amino acids [2] [3] [4] [5].

Nori (*Pyropia yezoensis*), a soft and flavorful edible seaweed, is cultivated on the coast of the Ariake Sea in Fukuoka Prefecture, which has become a leading area of nori production in Japan [6] through use of the pole system cultivation method [7]. In recent years, low-quality faded nori has also been harvested, even in cultivation systems that involve the freezing of seedlings; this production method is relatively stable. Some of this faded nori is disposed of in the sea, with negative impacts on fisheries, or is incinerated without being bid [8]. Nori contains organic minerals (carbon, N, phosphoric acid, potash, lime, and bittern) and trace elements [iron, zinc, copper, chromium, molybdenum, manganese,

selenium, and iodine (I)]. Nori is rich in protein, I, dietary fiber, and several vitamins, including folic acid and vitamins B12 and K [9]. Liquid fertilizers derived from natural sources such as seaweeds are viable alternative fertilizers for agricultural crop production due to their high levels of organic matter, micro- and macro-elements, vitamins, fatty acids, and growth regulators. To promote the development of fermented nori liquid fertilizer, the efficacy of nori as fertilizer must be evaluated.

In this study, we focused on the use of fermented nori as fertilizer in an effort to develop a method to produce fermented nori liquid fertilizer with high mineral and I content. In recent years, seaweed extracts have become commercially available for use as liquid fertilizers [10]. The use of organic liquid fertilizers on economically important crops, such as komatsuna (*Brassica rapa* var. wakana komatsuna), has gained interest. Komatsuna is one of the most common vegetables in Japan. It belongs to the cabbage family and is known worldwide as Japanese mustard spinach. Like other members of the Cruciferae family, komatsuna has high nutritional value [11].

To examine the growth promotion or inhibitory effects of fermented nori seaweed liquid fertilizers (SLFs), komatsuna plants were grown in vermiculite, which lacks microorganisms. Under microorganism-free conditions, the organic matter in the SLFs is not easily decomposed and mineralized and, consequently, these organic materials cannot be absorbed directly by the plants. The objective of this study was to observe the effects of four types of fermented nori SLFs on germination, plant growth characteristics, SPAD values, and nutrient content (especially I content) of komatsuna plants cultivated in vermiculite, which lacks nutrients and microorganisms.

2. Materials and Methods

2.1. Production of Fermented Nori SLFs

Experimental materials included faded waste nori, molasses (SPOON SUGAR Co. Ltd., Hyogo, Japan), well water, and the microbial material BASE EIGHT (Sunpowers Co. Ltd., Nagasaki, Japan). Thirty-three liters of well water, 7 kg nori (unwashed or washed with well water), 2.5 L molasses, and 2.5 L BASE EIGHT (total volume 45 L) were added to a 50-L fermentation tank (MH-50; Suico Co. Ltd., Hyogo, Japan) and mixed well. The nori was fermented aerobically and anaerobically. Under aerobic fermentation, aeration was provided by a Tetra air pump (OX-60; Spectrum Brands Japan, Kanagawa, Japan). Under anaerobic fermentation, stirring was performed once each day for 5 min. An electric blanket and an insulating blanket were wrapped around the fermentation tank, and the temperature was maintained at approximately 30˚C. Fermentation was carried out for 3 months from April to July of 2016. Four types of SLF were produced: anaerobically fermented unwashed nori (SLF1), aerobically fermented unwashed nori (SLF2), anaerobically fermented washed nori (SLF3), and aerobically fermented washed nori (SLF4).

2.2. Measurement of Germination Rates

Fifty komatsuna seeds were germinated in a petri dish on three layers of filter paper (85 mm) and one Tanepita germination test sheet (Fujihara Industry, Tokyo, Japan) according to the manufacturer's instructions. Then, 10 mL undiluted SLFs and 10-, 100-, 200-, 300-, and 400-fold dilutions of SLFs were added gently so as to not displace the seeds. The SLFs were diluted with distilled H_2O. Six incubation treatments were applied to each SLF, with three replications. A control treatment (distilled H_2O) was included, with three replications. The petri dishes were placed in an incubator at 25˚C for 4 days. The number of germinated seeds in each petri dish was counted at 2 days (48 h), 3 days (72 h), and 4 days (96 h) after sowing.

After counting the number of germinated komatsuna seeds, the relative germination rate (RGR) was calculated using the following equation;

$$\text{Relative germination rate } (RGR) = \frac{\text{Number of germinated seeds on each SLF dilution}}{\text{Average number of germinated seeds on the control}} \times 100$$

2.3. Cultivation of Komatsuna Plants

The wakana komatsuna variety was used in all experiments. Three types of experiment were conducted in pots at Kyushu University's Phytotron (25˚C and 75% RH). In Experiment 1, six treatments were tested with a completely randomized design, with three replications. Treatments were basal application of SLF1, SLF2, SLF3, SLF4, or ½-strength modified Hoagland nutrient (MHN) [12] solution containing NH_4NO_3or $NaNO_3$. One-liter pots were filled with vermiculite, and 0.6 L SLF or ½-strength MHN solution was added. The compositions of 1/2 strength MHN are 5 mM $NaNO_3$ or 2.5 mM NH_4NO_3, 3 mM K_2SO_4, 0.5 mM KH_2PO_4, 1 mM $CaC1_2$, 1mM $MgSO_4$, 2.5 mM NaFe EDTA, 16 μM $CuSO_4$, 4.6 μM $MnSO_4$, 3.6 μM $ZnSO_4$, 2.34 μM H_3BO_3 and 2.5 μM MoO_3. Five holes were made in the vermiculite in each pot and three seeds were sown in each hole, for a total of 15 seeds sown in each pot. The plants were cultivated in a controlled-environment room (25˚C and 75% relative humidity) for 5 weeks. The seedlings were thinned at 10 days after sowing to maintain five plants per pot. SLFs or ½-strength MHN containing NH_4NO_3 or $NaNO_3$ were applied when the pot weight decreased to 200 - 300 g. Total N and K of SLFs and ½-strength MHN solution were adjusted to 7.5 and 3.0 mM, respectively, to provide the same levels of N and K nutrients. The pH of SLFs and ½-strength MHN solution were adjusted 6.5 by using 1N NaOH or $1NH_2SO_4$. Experiment 1 was conducted from January to February of 2017.

For Experiment 2, the overall experimental design and cultivation methods were the same as for Experiment 1. In total, 10 treatments were used in Experiment 2. Treatments included the basal treatment and the basal treatment plus foliar application of SLF1, SLF2, SLF3, SLF4, or ½-strength MHN containing NH_4NO_3. The pH of SLFs and ½-strength MHN solution were adjusted 6.5 by using 1N NaOH or $1NH_2SO_4$. N and K concentrations were adjusted to 5 and 3

mM, respectively, to provide the same levels of N and K nutrients. Experiment 2 was conducted from April to May of 2017. Foliar spray applications of 10 mL of 10-fold dilutions of original solution of SLF1, SLF2, SLF3, SLF4, and ½-strength MHN were done at 3rd and 4th week after sowing.

For Experiment 3, the overall experimental design and cultivation methods were the same as for Experiment 1. In total, 13 treatments were used in Experiment 3. Treatments included foliar application of 10-, 20-, and 50-fold dilutions of original solution of SLF1, SLF2, SLF3, and SLF4; deionized water as a control treatment; and ¼-strength MHN containing $NaNO_3$ as a basal treatment and for irrigation. The compositions of ¼-strength MHN are half amount of nutrients in ½ strength MHN. Foliar spray applications were done at 5-day intervals from 11 days after sowing. Irrigation was applied when the pot weight decreased to 200 - 300 g with ¼-strength MHN. Experiment 3 was conducted from June to July of 2017.

2.4. Evaluation of the Effects of SLFs on Plant Growth and SPAD Values of Komatsuna Plants

Plant growth characteristics (leaf number and leaf length) were recorded at 3-day intervals beginning 10 days after sowing. Soil and plant analysis development (SPAD) values were measured using a chlorophyll meter (SPAD-502, Konica Minolta Sensing Inc., Osaka, Japan) at 3-day intervals beginning 14 days after sowing. The final collection of data on plant growth characteristics was performed for all treatments at 1 day before harvest. After 35 days, the plants were harvested by cutting at the cotyledonary node. The harvested plants were washed three times with deionized H_2O to remove the nutrients contained in the foliar spray. Plants were freeze dried for 72 h and the dry weights (g) of shoots were determined.

2.5. Evaluation of the Effects of SLFs on Total N, P, K, Ca, Mg, and Na Uptake

Freeze-dried shoot samples were cut into small pieces and ground to a fine powder using a Cyclotec 1093 sample mill (100 - 120 mesh, Tecator AB, Hoedanaes, Sweden). The fine powders were digested using the salicylic acid-H_2SO_4-hydrogen peroxide (H_2O_2) digestion method [13] as preparation for determining total N and phosphorus (P) contents, and the HNO_3 digestion method [14] as preparation for determining total potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) contents. The total N content was determined using the indophenol method [15]; total P was determined by the ascorbic acid method [16]; and K, Na, Ca, and Mg contents were determined using an atomic absorption spectrophotometer (Z-5300, Hitachi, Tokyo, Japan).

2.6. Evaluation of the Effects of SLFs on the I Content of Komatsuna Plants

Freeze-dried shoot samples were cut into small pieces and ground to a fine

powder using a Cyclotec 1093 sample mill (100 - 120 mesh, Tecator AB, Hoedanaes, Sweden). To determine the I content, 0.1-g plant samples were extracted with 10 mL 0.5% tetramethylammonium hydroxide [$(CH_3)_4OH$, TMAH] according to the extraction method of Fecher *et al.* [17] and Radlinger and Heumann [18]. The extract was placed in a 50-mL metal-free plastic centrifuge tube (SCP Science, equivalent to DigiTubes Goods) and incubated at 60˚C for 24 h in an oven. After cooling, 40 mL pure H_2O was added and the samples were centrifuged for 10 min at 1972 ×g using a swinging bucket rotor (KOKUSAN H, 80F equivalent). The extracted samples were then filtered through a 0.2-μm acetate cellulose membrane filter (Toyo Roshi, Tokyo, Japan). Finally, 200 μL 2 $\mu g \cdot mL^{-1}$ tellurium solution was added to 20 mL filtrate. The total I content was determined using an Agilent 7500ce inductively coupled plasma mass spectrometer (ICP-MS, Agilent Technologies, Inc., Tokyo, Japan).

2.7. Data Analysis

Data were statistically analyzed using Statistix 8 software (Analytical Software, Tallahassee, FL, USA) and mean values were compared for statistical significance using Tukey's HSD test at $P < 0.05$.

3. Results

3.1. Physiochemical Properties of Fermented Nori SLFs

The nutrient contents of the four types of fermented nori SLF examined in this study are shown in **Table 1**. The macronutrients contained in the SLFs included N, K, P, Ca, Mg, and Na. SLF2 had significantly higher N and Mg contents (80.31 and 27.15 mM, respectively) than SLF1 and SLF3, and higher N and Mg contents (76.35 mM and 26.95 mM, respectively) than SLF4. The highest K and Na contents were found in SLF1 and SLF2 obtained from unwashed nori, followed by SLF3 and SLF4 obtained from washed nori with reduced salt content. The SLFs also contained organic ions, such as ammonia nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), phosphate ($H_2PO_4^+$), chlorine (Cl^-), and sulfate (SO_4^{2-}), with different pH and EC values (**Table 2**). SLF1 and SLF3 had significantly lower NH_4-N and NO_3-N contents than did SLF2 and SLF4. The highest Cl^- and SO_4^{2-} contents were detected in SLF1 and SLF2 obtained from unwashed nori, followed by SLF3 and SLF4 obtained from washed nori. The colors of the SLFs were evaluated visually; SLF1 and SLF3 were reddish brown, and SLF2 and SLF4 were dark brown. pH and EC values were determined at room temperature using pH and EC meters.

3.2. Effects of SLFs on Komatsuna Seed Germination

We determined the relative germination ratios (RGRs) of komatsuna seeds treated with various dilutions of the four SLFs (**Table 3**). Treatment of seeds with 10- and 100-fold dilutions of SLF2, SLF3, and SLF4, but not SLF1, suppressed the RGR during the first 2 days after sowing (DAS), but gradually

Table 1. Concentration of macronutrients in fermented seaweed liquid fertilizers (SLFs).

SLFs	N	P	K	Ca	Mg	Na
	Concentration (mM)					
SLF1	62.00 ± 0.84	3.74 ± 0.08	72.13 ± 0.77	16.34 ± 0.37	25.92 ± 0.41	53.72 ± 1.09
SLF2	80.31 ± 2.70	3.74 ± 0.08	76.35 ± 1.92	16.34 ± 0.37	27.15 ± 0.41	51.22 ± 0.33
SLF3	58.50 ± 1.45	3.26 ± 0.16	72.51 ± 0.90	16.97 ± 0.25	21.19 ± 0.21	15.22 ± 1.30
SLF4	76.35 ± 1.18	2.91 ± 0.12	68.42 ± 1.41	15.84 ± 0.37	26.95 ± 0.21	48.61 ± 0.11

Total concentration of N, P, K, Ca, Mg of SLFs were measured by spectrophotometer after digestion by salicylic-sulfuric acid digestion method. Data show mean value ± standard deviation (SD) (n-3).

Table 2. Concentration of inorganic ions, pH and EC of SLFs.

SLFs	NH_4^+	NO_3^-	$H_2PO_4^-$	Cl^-	SO_4^{2-}	pH	EC
	Concentration (mM)						(s/m)
SLF1	5.34 ± 0.99	0.00 ± 0.00	1.44 ± 0.03	1.90 ± 0.00	0.19 ± 0.00	5.30 ± 0.01	1.79 ± 0.01
SLF2	23.95 ± 0.53	0.12 ± 0.04	1.41 ± 0.00	2.05 ± 0.00	0.16 ± 0.00	6.76 ± 0.01	2.03 ± 0.01
SLF3	2.71 ± 0.93	0.00 ± 0.00	1/46 ± 0.01	0.99 ± 0.00	0.11 ± 0.00	5.16 ± 0.01	1.47 ± 0.00
SLF4	19.72 ± 0.63	0.09 ± 0.04	0.95 ± 0.02	1.23 ± 0.00	0.09 ± 0.00	4.76 ± 0.01	1.72 ± 0.00

Ammonia, nitrate and phosphate of SLFs were measured by spectrophotometer. Chlorine and sulphate were measured by ion chromatography. Data show mean value ± SD (n = 3).

Table 3. Effect of different dilution of SLFs on the relative germination ratio (RGR) of komatsuna.

DAS	SLFs	1D	10 D	100 D	200 D	300D	400D
2	SLF1	0 ± 0	69 ± 2	90 ± 3	101 ± 1	99 ± 3	102 ± 4
	SLF2	0 ± 0	77 ± 3	94 ± 3	96 ± 4	92 ± 5	95 ± 4
	SLF3	0 ± 0	60 ± 3	83 ± 2	90 ± 1	94 ± 7	92 ± 3
	SLF4	0 ± 0	63 ± 2	83 ± 3	95 ± 2	95 ± 2	101 ± 1
3	SLF1	0 ± 0	92 ± 2	100 ± 3	103 ± 1	104 ± 2	104 ± 0
	SLF2	0 ± 0	95 ± 4	92 ± 3	104 ± 2	105 ± 1	104 ± 1
	SLF3	0 ± 0	71 ± 3	92 ± 2	104 ± 1	104 ± 1	104 ± 2
	SLF4	0 ± 0	88 ± 3	92 ± 4	104 ± 0	102 ± 2	103 ± 2
4	SLF1	0 ± 0	100 ± 3	100 ± 3	99 ± 1	99 ± 1	99 ± 1
	SLF2	0 ± 0	95 ± 3	98 ± 2	100 ± 0	100 ± 0	99 ± 1
	SLF3	0 ± 0	96 ± 3	94 ± 2	100 ± 0	99 ± 1	99 ± 1
	SLF4	0 ± 0	98 ± 2	98 ± 2	100 ± 0	98 ± 2	99 ± 1

Data show mean value ± SD. DAS and D mean day after sowing and dilution ratio, respectively.

increased the RGR at 3 and 4 DAS. The seeds treated with 200-, 300-, and 400-fold SLF dilutions exhibited increased RGR values at 3 and 4 DAS. At 4 DAS, the RGRs of seeds treated with 10-, 100-, 200-, 300-, and 400-fold SLF di-

lutions showed no differential effect. The seeds treated with undiluted SLFs did not germinate by 4 DAS. These results showed that high SLF concentrations suppressed the RGR during the early incubation period, but the RGR increased by 4 DAS. Therefore, dilution of the SLFs by at least 10-fold was required to not inhibit komatsuna seed germination. Additionally, SLF1 may promote komatsuna seed germination.

3.3. Effects of SLFs on Plant Growth Characteristics and SPAD Values (Experiment 1)

The plant growth characteristics and SPAD values of plants treated with basal applications of SLFs or ½-strength modified Hoagland solution (MHN) [12] differed significantly (**Table 4**). Plants treated with ½-strength MHN containing different N sources had higher leaf numbers, leaf lengths, and shoot dry weights than plants treated with the SLFs. Moreover, the growth parameters differed significantly among the SLFs. Plants treated with SLF2 and SLF4 produced significantly higher leaf numbers, leaf lengths, and shoot dry weights than plants treated with SLF1 or SLF3. Treatment with SLF4 or SLF2 produced significantly higher SPAD values than treatment with SLF1 or SLF3. By contrast, treatment with ½-strength MHN produced significantly lower SPAD values than treatment with the SLFs.

3.4. Effects of SLFs on N, P, K, Ca, and Mg Contents (Experiment 1)

The shoots of plants treated with the SLFs and ½-strength MHN differed significantly in their N, P, K, Ca, and Mg contents (**Table 5**). Application of ½-strength MHN containing different N sources produced the highest N, P, and K contents compared with those produced by the SLFs. The N, P, and Mg contents of plants treated with the SLFs did not differ. However, plants treated with SLF2 or SLF4 had significantly higher K contents than plants treated with SLF1 or SLF3. In contrast to K content, SLF1 treatment produced significantly higher Ca and Mg contents than ½-strength MHN. The Ca content of SLF1-treated plants differed from that of SLF2- and SLF4-treated plants, but not from that of SLF3-treated plants.

3.5. Effects of Basal Treatments and Basal Plus Foliar Application Treatments on Plant Growth Characteristics and SPAD Values (Experiment 2)

Plant growth characteristics and SPAD values did not differ significantly between the basal treatments and the basal plus foliar application treatments with the SLFs or ½-strength MHN (**Figure 1**); however, the effects on the growth parameters differed among the SLFs and ½-strength MHN. The plants treated with ½-strength MHN solution containing NO_3-N had higher leaf numbers, leaf lengths, and shoot dry weights than plants treated with the SLFs. Among the SLFs, treatment with SLF2 produced significantly higher leaf numbers, leaf lengths, and shoots dry weights, but did not affect the SPAD values. Plants treated

Table 4. Effect of SLFs basal and irrigation on leaf number, leaf length, shoot DW and SPAD value of komatsuna in Expt-1.

Treatments	Leaf number (plant^{-1})	Leaf length (cm)	Shoot DW (g·pot^{-1})	SPAD value
1/2MHN ($NaNO_3$)	6.16 ± 0.03 a	18.25 ± 0.19 a	3.75 ± 0.20 a	36.97 ± 0.46 c
1/2MHN (NH_4NO_3)	6.01 ± 0.05 a	19.17 ± 0.61 a	4.18 ± 0.12 a	37.30 ± 0.64 c
SLF1	4.24 ± 0.00 d	9.71 ± 0.20 c	0.71 ± 0.04 c	41.17 ± 1.42 b
SLF2	5.00 ± 0.07 b	12.25 ± 0.30 b	1.90 ± 0.37 b	43.29 ± 0.08 ab
SLF3	4.21 ± 0.12 d	9.94 ± 0.14 c	0.80 ± 0.04 c	41.71 ± 0.69 b
SLF4	4.61 ± 0.15 c	12.51 ± 0.42 b	1.49 ± 0.05 b	44.61 ± 0.59 a

Data show mean value ±SD (n = 3). The same letters are not significantly different at $P < 0.05$ (Tukey's test) in each column.

Table 5. Effect of basal and irrigation of SLFs on N, P, K, Ca and Mg content (%) of konmatsuna plant in Expt 1.

Treatments	N	P	K	Ca	Mg
1/2 H $NaNO_3$	4.22 ± 0.18 a	1.31 ± 0.07 a	5.37 ± 0.16 b	2.84 ± 0.05 bc	0.28 ± 0.05 b
1/2 H NH_4NO_3	3.89 ± 0.16 ab	1.45 ± 0.02 a	6.70 ± 0.11 a	2.78 ± 0.01 bc	0.29 ± 0.00 b
SLF1	3.37 ± 0.14 b	0.36 ± 0.01 b	2.63 ± 0.22 d	3.44 ± 0.02 a	0.40 ± 0.04 a
SLF2	3.52 ± 0.29 b	0.51 ± 0.05 b	4.07 ± 0.50 c	2.46 ± 0.17 c	0.33 ± 0.03 ab
SLF3	3.66 ± 0.27 ab	0.36 ± 0.05 b	3.01 ± 0.28 d	3.10 ± 0.22 ab	0.32 ± 0.02 ab
SLF4	3.49 ± 0.14 b	0.42 ± 0.02 b	4.06 ± 0.30 c	2.68 ± 0.10 c	0.31 ± 0.03 ab

Data show mean value ± SD (n = 3). The same letters in each column are not significantly different at $P < 0.05$ (Tukey's test).

with the SLFs had higher SPAD values than those treated with ½-strength MHN; in particular, treatment with SLF2 and SLF3 produced significantly higher SPAD values.

3.6. Effects of Basal Treatments and Basal Plus Foliar Application Treatments on N, P, K, Ca, Mg, and Na Contents (Experiment 2)

The effects of SLFs and ½-strength MHN solution on the N, P, K, Ca, Mg, and Na contents of shoots did not differ significantly between basal treatments and basal plus foliar application treatments (Table 6). Treatment with SLF4 resulted in higher N content than treatment with SLF1, SLF2, and ½-strength MHN; the second-highest N content was generated by treatment with SLF1. Plants treated with SLFs had significantly lower P and K contents than those treated with ½-strength MHN. The Mg content was the highest in plants treated with SLF1 or SLF4, and lowest in those treated with ½-strength MHN. The Na content differed to a highly significant degree between the plants treated with SLFs and those treated with ½-strength MHN.

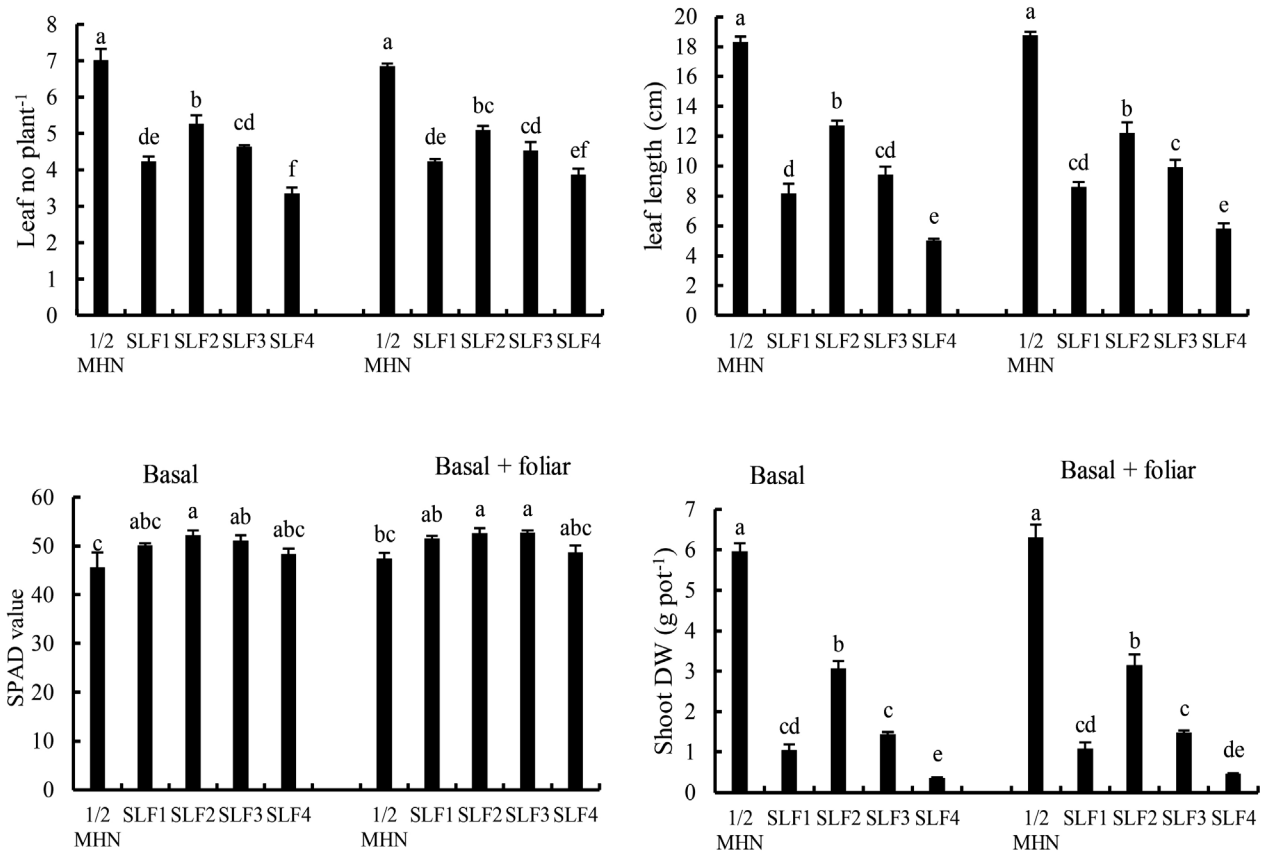

Figure 1. Effect of basal application and basal + foliar application of SLFs on leaf number, leaf length, shoot dry weight and SPAD value of komatsuna in Expt. 2. The same letters in each application are not significantly different at $P < 0.05$ (Tukey's test).

Table 6. Effect of SLFs on N, P, K, Ca and Mg content (%) of konmatsuna in Expt 2.

Treatments	N	P	K	Ca	Mg	Na
(Basal)						
1/2 MHN	2.38 ± 0.12 d	0.91 ± 0.01 a	6.11 ± 0.15 a	2.65 ± 0.12 ab	0.46 ± 0.48 cd	0.011 ± 0.00 ab
SLF1	3.44 ± 0.14 c	0.13 ± 0.01 b	1.38 ± 0.03 c	2.66 ± 0.09 ab	0.62 ± 0.05 a	0.008 ± 0.00 abc
SLF2	1.68 ± 0.06 e	0.15 ± 0.01 b	1.86 ± 0.16 b	1.78 ± 0.03 c	0.44 ± 0.01 d	0.007 ± 0.00 bc
SLF3	2.59 ± 0.15 d	0.15 ± 0.01 b	1.61 ± 0.02 bc	2.49 ± 0.08 b	0.51 ± 0.03 abcd	0.013 ± 0.00 bc
SLF4	5.56 ± 0.24 a	0.15 ± 0.00 b	0.96 ± 0.04 d	2.92 ± 0.06 a	0.57 ± 0.03 abc	0.002 ± 0.00 c
(Basal + foliar)						
1/2 MHN	2.38 ± 0.16 d	0.90 ± 0.07 a	6.03 ± 0.19 a	2.70 ± 0.18 ab	0.48 ± 0.03 bcd	0.010 ± 0.00 ab
SLF1	3.52 ± 0.41 c	0.16 ± 0.01 b	1.41 ± 0.07 c	2.60 ± 0.07 a	0.58 ± 0.01 ab	0.014 ± 0.00 a
SLF2	1.52 ± 0.06 e	0.14 ± 0.01 b	1.57 ± 0.10 bc	1.66 ± 0.06 c	0.41 ± 0.04 d	0.007 ± 0.00 bc
SLF3	2.64 ± 0.03 d	0.16 ± 0.01 b	1.82 ± 0.10 b	2.49 ± 0.04 b	0.51 ± 0.02 abcd	0.013 ± 0.00 a
SLF4	5.01 ± 0.16 b	0.19 ± 0.04 b	0.89 ±0.06 d	2.78 ± 0.03 ab	0.58 ± 0.01 ab	0.002 ± 0.00 c

Data show mean value ± SD (n = 3). The same letters in each column are not significantly different at $P < 0.05$ (Tukey's test).

3.7. Effects of Foliar Application of Different SLF Dilutions on Plant Growth Characteristics and SPAD Values (Experiment 3)

No significant difference was observed in the effects of foliar applications of different SLF dilutions on plant growth characteristics or SPAD values compared with the control (distilled H_2O) foliar treatment (Table 7).

3.8. Effects of Foliar Application of Different SLF Dilutions on N, P, K, Ca, Mg, and Na Uptake (Experiment 3)

No significant difference was observed in the effects of foliar applications of different SLF dilutions on N, P, K, Ca, or Mg uptake in shoots compared with the control foliar treatment (Table 8); however, Na uptake differed significantly between the treatments with different SLF dilutions and the control treatment (Figure 2). The plants treated with different dilutions of SLF1 and SLF2 (produced from unwashed nori) exhibited higher Na uptake than plants treated with SLF3 or SLF4 (produced from washed nori) and the control treatment.

3.9. Effects of SLFs on I Content (Experiment 3)

Plants treated with foliar applications of dilutions of the four SLFs exhibited significantly increased I content relative to the H_2O-treated control plants (Figure 2). Among the four SLFs, foliar application of SLF1 dilutions resulted in significantly higher I content (21.68 $\mu g \cdot g^{-1}$) than foliar application of dilutions of the other SLFs or the control.

Table 7. Effect of foliar application of SLFs on leaf number, leaf length, shoot dry weight and SPAD value of komatsuna in Expt. 3.

Foliar application	Dilution ratio	Leaf no ($plant^{-1}$)	leaf length (cm)	Shoot DW ($g \cdot pot^{-1}$)	SPAD value
H_2O	—	5.87 ± 0.25 ab	14.61 ± 0.11 a	3.75 ± 0.18 ab	40.93 ± 0.25 a
SLF1	10	5.61 ± 0.08 b	14.19 ± 0.39 a	3.74 ± 0.12 ab	40.11 ± 0.60 a
SLF1	20	5.69 ± 0.10 ab	15.35 ± 0.43 a	3.72 ± 0.20 ab	39.93 ± 0.91 a
SLF1	50	5.58 ± 0.16 ab	14.37 ± 0.24 a	3.61 ± 0.17 ab	40.35 ± 0.45 a
SLF2	10	5.97 ± 0.12 ab	15.36 ± 0.50 a	3.92 ± 0.06 a	39.46 ± 0.24 a
SLF2	20	6.01 ± 0.01 ab	15.01 ± 0.11 a	3.82 ± 0.08 ab	39.47 ± 0.63 a
SLF2	50	5.91 ± 0.08 ab	15.25 ± 0.38 a	3.76 ± 0.07 ab	40.13 ± 0.39 a
SLF3	10	5.83 ± 0.13 ab	14.27 ± 0.71 a	3.51 ± 0.11 ab	39.62 ± 1.06 a
SLF3	20	6.04 ± 0.04 a	15.01 ± 0.19 a	3.44 ± 0.06 ab	40.15 ± 0.53 a
SLF3	50	5.99 ± 0.15 ab	14.15 ± 0.11 a	3.38 ± 0.21 ab	40.55 ± 0.31 a
SLF4	10	5.99 ± 0.05 ab	14.67 ± 0.17 a	3.69 ± 0.08 ab	40.05 ± 0.78 a
SLF4	20	5.94 ± 0.04 ab	14.13 ± 0.19 a	3.58 ± 0.28 ab	40.63 ± 0.55 a
SLF4	50	5.93 ± 0.05 ab	14.78 ± 0.32 a	3.33 ± 0.19 b	40.23 ± 0.60 a

Data show mean value ± SD (n = 3). The same letters in each column are not significantly different at $P < 0.05$ (Tukey's test). All komatsuna plants were cultivated by using 1/4MHN as basal application and irrigation.

Table 8. Effect of foliar application of SLFs on N, P, K, Ca, Mg uptake (mg·pot^{-1}) of komatsuna in Expt.3.

Treatments	Dilution	N	P	K	Ca	Mg
H_2O	—	57.07 ± 0.01 ab	21.97 ± 0.03 ab	98.30 ± 0.06 ab	63.45 ± 0.04 ab	10.13 ± 0.01 abc
SLF1	10	58.31 ± 0.07 ab	22.28 ± 0.02 ab	100.77 ± 0.08 ab	68.75 ± 0.12 a	10.65 ± 0.02 abc
SLF1	20	59.62 ± 0.12 a	23.33 ± 0.03 a	107.20 ± 0.26 a	64.38 ± 0.05 ab	10.80 ± 0.01 ab
SLF1	50	55.87 ± 0.10 ab	21.41 ± 0.05 ab	104.03 ± 0.22 ab	62.11 ± 0.13 ab	10.77 ± 0.03 ab
SLF2	10	57.93 ± 0.02 ab	23.12 ± 0.01 a	111.96 ± 0.02 a	65.77 ± 0.06 ab	11.51 ± 0.01 a
SLF2	20	58.33 ± 0.02 ab	22.78 ± 0.01 a	108.16 ± 0.07 a	62.81 ± 0.06 ab	11.01 ± 0.01 abc
SLF2	50	56.28 ± 0.11 ab	21.25 ± 0.01 ab	107.29 ± 0.16 a	62.94 ± 0.07 ab	10.08 ± 0.01 abc
SLF3	10	53.59 ± 0.12 ab	20.61 ± 0.03 ab	94.37 ± 0.21 ab	59.86 ± 0.15 ab	9.90 ± 0.02 abc
SLF3	20	51.90 ± 0.01 ab	21.04 ± 0.02 ab	100.72 ± 0.08 ab	55.66 ± 0.01 b	9.18 ± 0.01 bc
SLF3	50	53.17 ± 0.05 ab	20.35 ± 0.02 ab	100.80 ± 0.15 ab	54.91 ± 0.03 b	8.61 ± 0.01 c
SLF4	10	57.54 ± 0.09 ab	22.00 ± 0.02 ab	108.13 ± 0.08 a	65.38 ± 0.12 ab	10.63 ± 0.02 abc
SLF4	20	54.96 ± 0.07 ab	21.30 ± 0.02 ab	98.36 ± 0.15 ab	60.56 ± 0.08 ab	9.47 ± 0.03 abc
SLF4	50	49.14 ± 0.06 b	18.95 ± 0.01 b	86.14 ± 0.05 b	58.36 ± 0.03 ab	9.08 ± 0.00 bc

Data show mean value ± SD (n = 3). The same letters in each column are not significantly different at P < 0.05 (Tukey's test). All komatsuna plants were cultivated by using 1/4 MHN as basal application and irrigation.

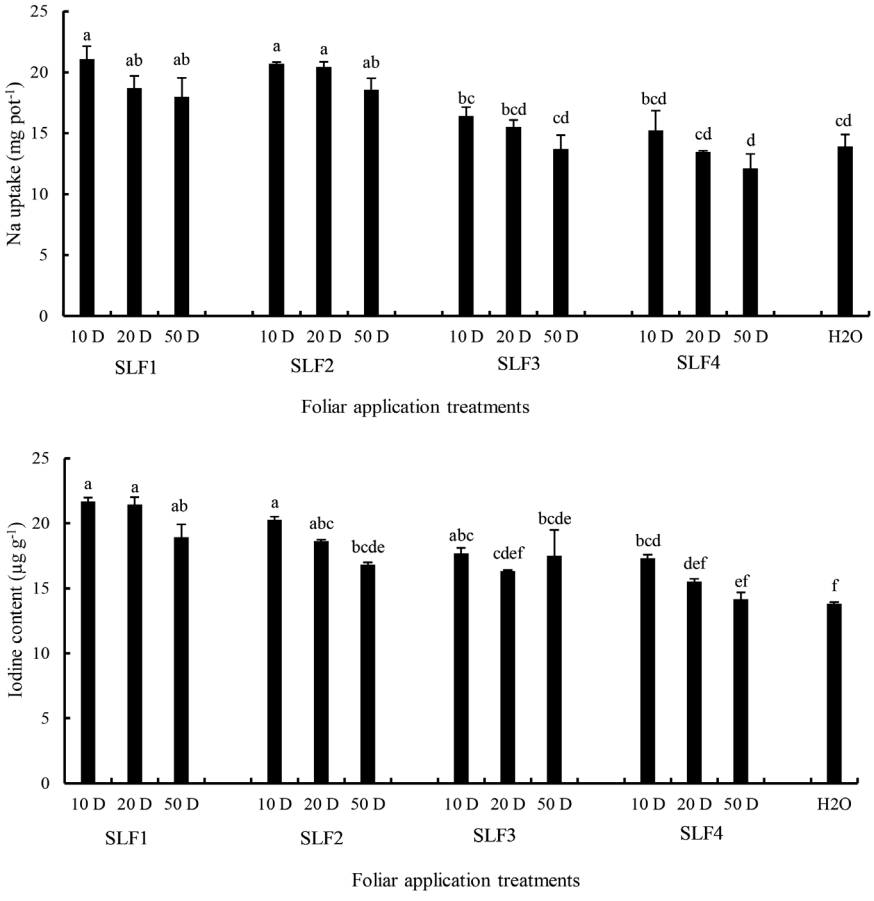

Figure 2. Effect of SLFs on Na uptake (mg·pot^{-1}) and Iodine content (μg·g^{-1}) of komatsuna in Expt-3. 1/4MHN was used as basal application and irrigation. The same letters in each parameter are not significantly different at P < 0.05 (Tukey's test).

4. Discussion

In this study, komatsuna seeds treated with 10- and 100-fold dilutions of SLF2, SLF3, and SLF4, but not SLF1, suppressed the RGR during 1 and 2 DAS, and gradually increased the RGR during 3 and 4 DAS. Seeds treated with the 200-, 300-, and 400-fold SLF dilutions had higher RGRs at 3 and 4 DAS. At 4 DAS, the RGRs of seeds treated with 10-, 100-, 200-, 300-, and 400-fold SLF dilutions showed no differential effect. The seeds treated with undiluted SLFs did not germinate by 4 DAS. Kalaivanan and Venkatesalu (2012) [19] reported that *Vigna mungo* seeds soaked in low concentrations of seaweed extracts showed higher rates of germination, whereas increasing concentrations of the extracts inhibited germination. Balamurugan.G. (2013) also reported that *Abelmoschus esculentus* seeds treated with seaweed (*Sargassum myryocystem*) extracts, which is prepared in the ratio 1:10 W/V, is an increase in rate of germination of seeds at lower concentration and reduces in rate of germination at higher concentration [20]. Our results showed that high SLF concentrations suppressed the RGR during the early incubation period, but the RGR was increased at 4 DAS. Therefore, dilution of the SLF extracts at least 10-fold is necessary so as to not inhibit komatsuna seed germination. Additionally, we found that SLF1 may enhance komatsuna seed germination.

Based on the results of Experiments 1 and 2, the growth characteristics of plants treated with SLFs were significantly suppressed compared with those of plants treated with ½-strength MHN, followed by those of plants treated with SLF2 and SLF4, even though the same concentrations of N and K were applied to each pot. This may be because SLF2 and SLF4 were produced from aerobic fermentation. Aerobic fermentation is a short and relatively stable process that occurs in the presence of sufficient oxygen; many low-molecular-weight organic substances are decomposed into carbon dioxide and inorganic matter. When aeration is sufficient, most nitrogen compounds contained in the material can be harvested as high-mineral-content liquid fertilizer. Treatment with SLF2 significantly increased plant growth characteristics compared with the other SLFs, but treatment with undiluted SLFs suppressed plant growth. In Experiment 3, the dry matter production of plants treated with foliar spray application of 10-fold diluted SLF2 was significantly higher than that produced by foliar application of 50-fold diluted SLF4, but did not differ significantly from the effects of the other SLFs. Furthermore, the color of all plant leaves treated with SLFs became darker and the SPAD values were higher than with ½-strength MHN treatment. This was likely due to P deficiency in plants under all SLF treatments, with P content markedly lower than in plants treated with ½-strength MHN (**Table 5** and **Table 6**). Seaweed fertilizer has been reported to be richer in K, but poorer in N and P, than farm manure [21].

In Experiment 1, the plants treated with ½-strength MHN containing different N sources exhibited the highest N, P, and K contents compared with those treated with the SLFs. N, P, and Mg contents did not differ among SLF treat-

ments. However, plants treated with SLF2 or SLF4 had significantly higher K contents than those treated with SLF1 or SLF3, possibly because SLF1 and SLF3 were produced by anaerobic fermentation. Because the oxygen supply is low under anaerobic fermentation, many low-molecular-weight organic substances are produced. As SLF is a good source of K, it helps regulate the water status of the plants, controls the opening and closing of stomata, and enhances photosynthesis and disease resistance. Treatment with SLF1 resulted in significantly higher Ca and Mg contents than treatment with ½-strength MHN. The Ca content of plants treated with SLF1 differed from that of plants treated with SLF2 or SLF4, but not from that of plants treated with SLF3. The Ca present in seaweed extracts facilitates enzyme activation, cell elongation, and cell stability [22]. SLF is a rich source of secondary nutrients, such as Mg; hence, it enhances photosynthesis, phloem export, root growth, and N metabolism [22].

In Experiment 2, the N, P, K, Ca, Mg, and Na contents of shoots differed significantly between plants receiving the basal treatment and those receiving the basal plus foliar application treatment with ½-strength MHN and SLFs. Treatment with SLF4 produced higher N content than SLF1, SLF2, or ½-strength MHN treatment; the second-highest N content was produced by SLF1 treatment. Treatment with SLFs produced significantly lower P and K contents than treatment with ½-strength MHN. The highest Mg content was obtained with SLF1 and SLF4 treatment, while the lowest value was obtained with ½-strength MHN. The Na contents of SLF-treated plants showed highly significant differences from those of ½-strength MHN-treated plants. A previous study showed that the application of a commercial extract of *E. maxima* to lettuce plants grown under optimal conditions improved yield and the concentrations of Ca, K, and Mg in the leaves [23]. The concentrations of Ca, K, and Mg in the lettuce leaves increased with increasing nutrient supply. In this study, the Ca and Mg contents of komatsuna leaves were increased by SLF1 (**Table 5** and **Table 6**). In Experiment 3, no significant difference in N, P, K, Ca, or Mg uptake in shoots was observed with foliar application of different SLF dilutions in combination with basal irrigation with ¼-strength MHN; however, I content differed significantly.

Na uptake differed significantly among SLF dilutions in Experiment 3. Foliar treatment with different dilutions of SLF1 and SLF2 caused more Na uptake than treatment with SLF3, SLF4, or ¼-strength MHN (**Figure 2**). This effect may correspond to whether washed or unwashed nori was used to make the extracts. SLF1 and SLF2 obtained from unwashed nori contained more salt than SLF3 and SLF4, which were produced from nori washed with fresh water, indicating that washing the nori can reduce the salt content of the fertilizer; however, when seawater is diluted and applied to agricultural land, abundant minerals are provided to crops, improving their quality [24].

In this study, the I content differed significantly among the plants treated with the different SLFs and with foliar applications of different SLF dilutions. Foliar application of different SLF dilutions significantly increased the I content com-

pared with the control foliar application (**Figure 2**). Among the foliar applications of different SLF dilutions, SLF1 significantly increased the I content compared with other SLFs and the control. Nori is rich in protein, I, dietary fiber, and several vitamins, including folic acid and vitamins B12 and K (Japan Food Standard Ingredient **Table 2** 015) [9]. The I content values for nori, wakame, and kombu were reported as 29.3 - 45.8 (avg. 36.9) mg/kg, 93.9 - 185.1 (avg. 139.7) mg/kg, and 241 - 4921 (avg. 2523.3) mg/kg, respectively [25] [26] [27] [28] [29]. According to the results of Experiments 1, 2, and 3, SLF1 has the greatest potential for use as liquid fertilizer because its Ca, Mg, Na, and I contents are higher of than those of the other SLFs and ½-strength MHN (Tables 5 and 6). Therefore, the increased growth, increased nutrient content, and higher I content of plants treated with SLF1 may be due to its higher macronutrient content (**Table 1**), and the higher ammonia nitrogen (NH_4-N), nitrate nitrogen (NO_3-N), phosphate ($H_2PO_4^+$), chlorine (Cl^-), and sulfate (SO_4^{2-}) contents (**Table 2**).

In our experiments with plants grown in vermiculite, the concentrations of nutrients, especially P, and the growth performance of plants treated with SLFs were lower than those of plants treated with Hoagland solution. As microorganisms were not present in the vermiculite, not only was the organic matter not mineralized, but the nutrients could not be absorbed by the plants. Because our experiments were performed in vermiculite, in which organic matter contained in the SLFs could not be decomposed, similar experiments should be carried out in soil containing microbes, which can degrade the organic materials in SLFs.

5. Conclusion

In this study, komatsuna seeds treated with 200-, 300-, and 400-fold dilutions of SLFs had the highest RGRs at 4 DAS. The seeds treated with undiluted SLFs did not germinate by 4 DAS, indicating that dilution of SLFs by 10-fold or more is required to prevent inhibition of germination and seedling growth. Although SLF1 suppressed seed germination during 1 and 2 DAS, seed germination increased gradually during 3 and 4 DAS. Among the SLFs, SLF1 showed the best results for N, Ca, Mg, Na, and I content and promoted germination of komatsuna seeds. Therefore, SLF1 with at least 10-fold dilution can be used as a liquid fertilizer in appropriate combination with chemical fertilizer as a basal treatment and as a foliar treatment for komatsuna production.

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References

[1] Karasahin. M. (2017) The Effects of Different Seaweed Doses on Yield and Nutritional Values of Hydroponic Wheatgrass Juice. *Turkish Journal of Agriculture—Food Science and Technology*, **5**, 226-230.

https://doi.org/10.24925/turjaf.v5i3.226-230.994

[2] Sharma, S.H.S., Lyons, G., McRoberts, C., McCall, D., Carmichael, E., Andrews, F., Swan, R., McCormack, R. and Mellon, R. (2012) Biostimulant Activity of Brown Seaweed Species from Strangford Lough: Compositional Analyses of Polysaccharides and Bioassay of Extracts Using Mung Bean (*Vigno mungo* L.) and Pak Choi (*Brassica rapa* Chinensis L.). *Journal of Applied Phycology*, **24**, 1081-1091. https://doi.org/10.1007/s10811-011-9737-5

[3] Latique, S., Chernane, H., Mansori, M. and El Kaoua, M. (2013) Seaweed Liquid Fertilizer Effect on Physiological and Biochemical Parameters of Bean Plant (*Phaesolus vulgaris* Variety Paulista) under Hydroponic System. *European Scientific Journal*, **9**, 174-191.

[4] Peinado, I., Giron, J., Koutsidis, G. and Ames, J.M. (2014) Chemical Composition, Antioxidant Activity and Sensory Evaluation of Five Different Species of Brown Edible Seaweeds. *Food Research International*, **66**, 34-44. https://doi.org/10.1016/j.foodres.2014.08.035

[5] Shahbazi, F., Seyyed Nejad, M., Salimi, A. and Gilani, A. (2015) Effect of Seaweed Extracts on the Growth and Biochemical Constituents of Wheat. *International Journal of Agriculture and Crop Sciences*, **8**, 283-287.

[6] Ministry of Agriculture, Forestry and Fisheries (2016) I. Production Structure and Employment Structure of Marine Fisheries, in Census of Fisheries. https://www.e-stat.go.jp/en/stat-search/files?page=1&query=Nori%20culture&layout=dataset&stat_infid=000031367043

[7] Kawamura, Y. (1998) Basic Manual of Nori Produce in the Pole System Cultivation. *Bulletin of Saga Prefectural Ariake Fisheries Research and Development Center*, **18**, 37-52.

[8] Akimoto, T. and Shimizu, N. (2005) The Measure of Effective Use of Low Quality *Porphyra lavgr*. *Bulletin of Fukuoka Fisheries and Marine Technology Research Center*, **15**, 123-132.

[9] MEXT 2015 Standard Tables of Food Composition in Japan (Seventh Revised Edition). http://www.mext.go.jp/en/policy/science_technology/policy/title01/detail01/sdetail01/sdetail01/1385122.htm

[10] Divya, K and Niranjana Reddi, B. (2017) Influence of Seaweed Liquid Fertilizer of *Sargassum wightii*, *Turbnearia arnata* on the Seed Germination, Growth and Productivity of Vegetable Crops. *Journal of Algal Biomass Utilization*, **8**, 37-43.

[11] Acikgoz, F.E., Adiloglu, A., Daglioglu, F., Adiloglu, S., Celikyurt, G. and Karakas, O. (2014) The Effect of Increasing Doses of Nitrogen (N) Application for Some Nutrient Elements, Vitamin C and Protein Contents of Komatsuna (*Brassica rapa var. perviridis*) Plant. *Bulgarian Journal of Agricultural Science*, **20**, 321-324.

[12] Haider, J., Hussain, A.K.M.A., Ikeda, M., Yamakawa, T. and Ishizuka, J. (1991) Effect of Nitrate Application on Growth, Nodulation, and Nitrogen Fixation of Nitrate-Tolerant Mutants of Soybean. *Soil Science and Plant Nutrition*, **37**, 521-529. https://doi.org/10.1080/00380768.1991.10415065

[13] Ohyama, T., Ito, M., Kobayashi, K., Araki, S., Yasuyoshi, S., Sasaki, O., Yamazaki, T., Soyama, K., Tanemura, R., Mizuno, Y. and Ikarashi, T. (1991) Analytical Procedures of N, P, K Contents in Plant and Manure Materials Using H_2SO_4-H_2O_2 Kjeldahl Digestion Method. *Bulletin of the Faculty of Agriculture—Niigata University*, **43**, 110-120.

[14] Niazi, S.B., Littlejohn, D. and Halls, D.J. (1993) Rapid Partial Digestion of Biological

Tissues with Nitric Acid for the Determination of Trace Elements by Atomic Spectrometry. *Analyst*, **118**, 821-825. https://doi.org/10.1039/an9931800821

[15] Cataldo, D.A., Schrader, L.E. and Youngs, V.L. (1974) Analysis by Digestion and Colorimetric Assay of Total Nitrogen in Plant Tissues High in Nitrate. *Crop Science*, **14**, 854-856. https://doi.org/10.2135/cropsci1974.0011183X001400060024x

[16] Murphy, J. and Riley, J. (1962) A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. *Analytica Chimica Acta*, **27**, 31-36.

[17] Fecher, P.A., Goldmann, I. and Nagengast, A. (1998) Determination of Iodine in Food Samples by Inductively Coupled Plasma Mass Spectrometry after Alkaline Extraction. *Journal of Analytical Atomic Spectrometry*, **13**, 977-982. https://doi.org/10.1039/a801671b

[18] Rädlinger, G. and Heumann, K.G. (1998) Iodine Determination in Food Samples Using Inductively Coupled Plasma Isotope Dilution Mass Spectrometry. *Analytical Chemistry*, **70**, 2221-2224. https://doi.org/10.1021/ac971308k

[19] Kalaivanan, C. and Venkatesalu, V. (2012) Utilization of Seaweed *Sargassum myriocystum* Extracts as a Stimulant of Seedlings of *Vigna mungo* (L.) Hepper. *Spanish Journal of Agricultural Research*, **10**, 466-470. https://doi.org/10.5424/sjar/2012102-507-10

[20] Balamurugan, G. (2013) Effect of Seaweed Liquid Fertilizer of *Sargassum myryocystem* of *Abelmoschus esculentus* (L.). *International Journal of Current Research and Development*, **1**, 33-37.

[21] Kingman, A.R. and Moore, J. (1982) Isolation, Purification and Quantification of Several Growth Regulating Substance in *Ascophyllum nodosum* (Phaeophyceae). *Botanica Marina*, **25**, 149-153. https://doi.org/10.1515/botm.1982.25.4.149

[22] Pramanick, B., Brahmachari, K., Ghosh, A. and Zodape, S.T. (2016) Effect of Seaweed Saps Derived from Two Marine Algae *Kappaphycus* and *Gracialaria* on Growth and Yield Improvement of Blackgram. *Indian Journal of Geo-Marine Sciences*, **45**, 789-794.

[23] Crouch, I.J., Beckett, R.P. and Van Staden, J. (1990) Effect of Seaweed Concentrate on the Growth and Mineral Nutrition of Nutrient Stress Lettuce. *Journal of Applied Phycology*, **2**, 269-272. https://doi.org/10.1007/BF02179784

[24] Harada, C., Serita, T. and Tanaka, A. (2006) The Influence of Drip Irigation with Sea Water on the Quality of Tomatoes. *Coastal Bioenvironment*, **8**, 51-62.

[25] Mabeau, S. and Fleurence, J. (1993) Seaweed in Food Products: Biochemical and Nutritional Aspects. *Trends in Food Science & Technology*, **4**, 103-107. https://doi.org/10.1016/0924-2244(93)90091-N

[26] Dawczynski, C., Schaefer, U., Leiterer, M., *et al.* (2007) Nutritional and Toxicological Importance of Macro, Trace, and Ultra-Trace Elements in Algae Food Products. *Journal of Agricultural and Food Chemistry*, **55**, 10470-10475. https://doi.org/10.1021/jf0721500

[27] MacArtain, P., Gill, C.I. and Brooks, M. (2007) Nutritional Value of Edible Seaweeds. *Nutrition Reviews*, **65**, 535-543. https://doi.org/10.1111/j.1753-4887.2007.tb00278.x

[28] Holdt, S. and Kraan, S. (2011) Bioactive Compounds in Seaweed; Functional Food Applications and Legislation. *Journal of Applied Phycology*, **23**, 543-597.

[29] Kikuchi, Y., Takebayashi, T. and Sasaki, S. (2008) Iodine Concentration in Current Japanese Food and Beverages. *Nippon Eiseigaku Zasshi*, **63**, 724-734. https://doi.org/10.1265/jjh.63.724